NIST Role in BioMetrology

Vincent L. Vilker
Chief, Biotechnology Division
NIST Visiting Committee Meeting
June 10, 2003

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Technology Administration, U.S. Department of Commerce

Overview

- Economic/Societal Areas of Impact
- Metrology Needs in Bioscience
- Current NIST Biometrology Activity
- Looking to the Future

Economic/Societal Areas of Impact

- ***Health Care***
- ***Food & Agrobiotechnology***
- ***Biomanufacturing***
- ***Homeland Security (Forensics & Biodefense)***
- ***International Trade***

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Areas of Impact: Health Care

- ***Pharmaceutical***
  - *Discovery*
  - *Validation*
- ***Diagnostic***
  - *Reagents*
  - *Devices*
- ***Delivery & Patient Care***
  - *Clinical Services*
  - *Patient Information Handling*
  - *Providers, Medical Plans & Insurance*

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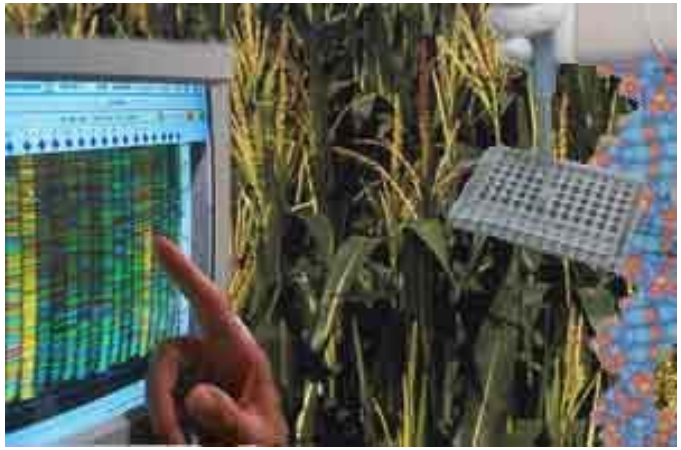
Areas of Impact: Food & Agbiotechnology

- ***Bio-based Production***
  - *Plant Crops*
  - *Non-plant Therapeutics*



- ***Bio-based Food Processing***
  - *Food Production*
  - *Flavors & Fragrances*

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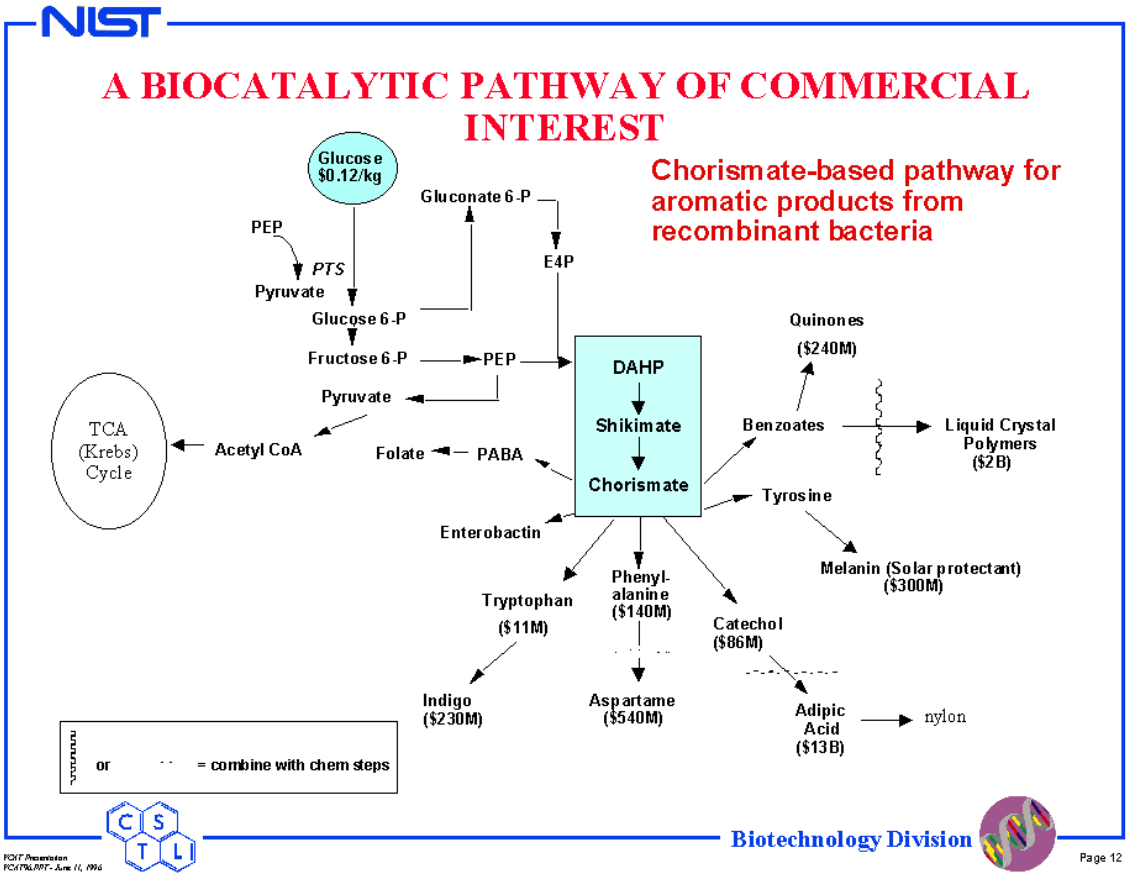
Areas of Impact: Biomanufacturing

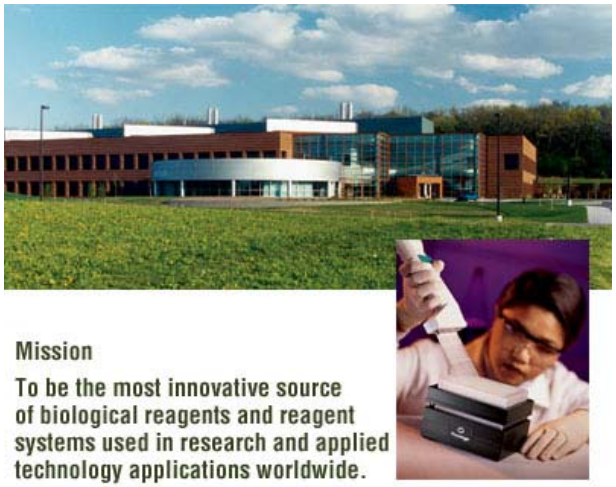
- ***Advanced Materials***
- ***Reagents***



Areas of Impact: Home Land Security (Forensics & Biodefense)

- ***Reagents***

Promega/NIST/FBI collaborate on finding new DNA-biomarkers



- ***Devices***

Hand-held immunoassay test strips

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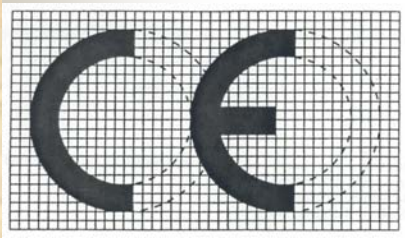
Areas of Impact: International Trade

- *In Vitro Diagnostics (IVD)*
- *Genetically Modified Crops (aka GMOs)*
- *Mycotoxin Contamination*
- *Bovine Spongiform Encephalopathy (BSE) & Prion-Related Diseases*

International Trade: EU IVD Directive

A New Driver:
EU IVD Directive to go into effect 2003

- Worldwide *in vitro* diagnostic device market is ~$20B;
- **>60% of European market is supplied by U.S.**



Stated Purpose of Directive

- Eliminate trade barriers *within Europe* by ensuring access to the entire EU market with one single product approval (CE Mark)

US IVD Manufacturers have requested that NIST develop internationally recognized reference methods and SRMs to meet the traceability requirement

Essential Requirements/Implementation

- IVD Calibrators and/or control materials must be traceable to ***"standards of a higher order"*** -- nationally/internationally recognized *certified reference materials*
- ***New*** IVD products ***must*** have mark by December 2003
- ***Existing*** IVD products may be sold without CE mark until December 2005

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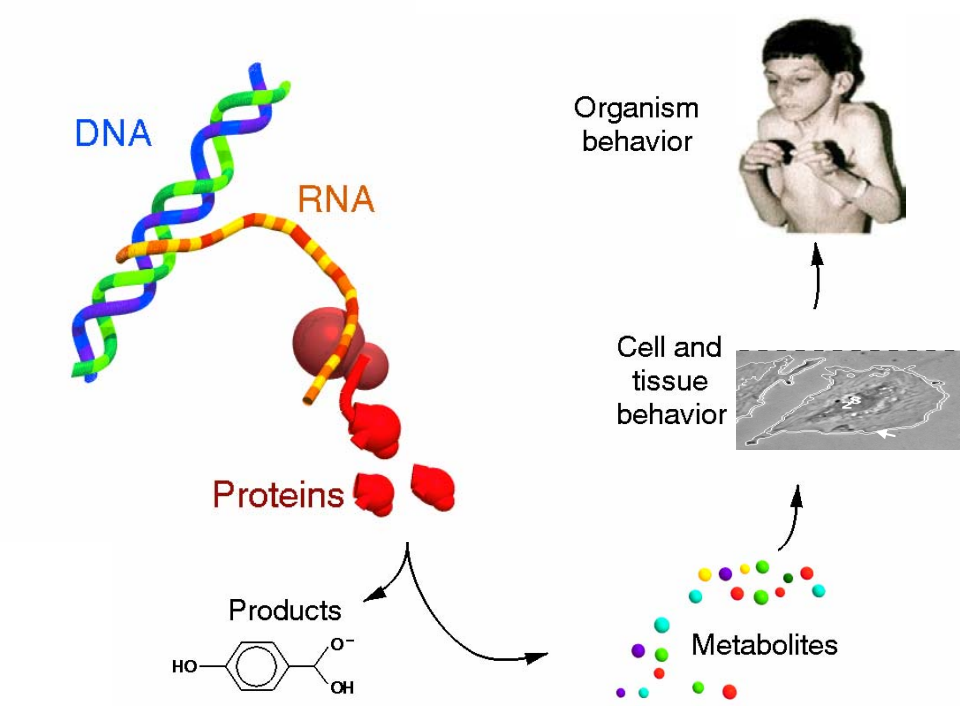
Metrology Needs in Bioscience

Biometrology refers to measurement and data activities that provide quantitative characterization of biology

- Gene Typing (Genomics)
- Gene Expression
- Proteins & Proteomics
- Cellular & Tissue Biology
- Bioinformatics



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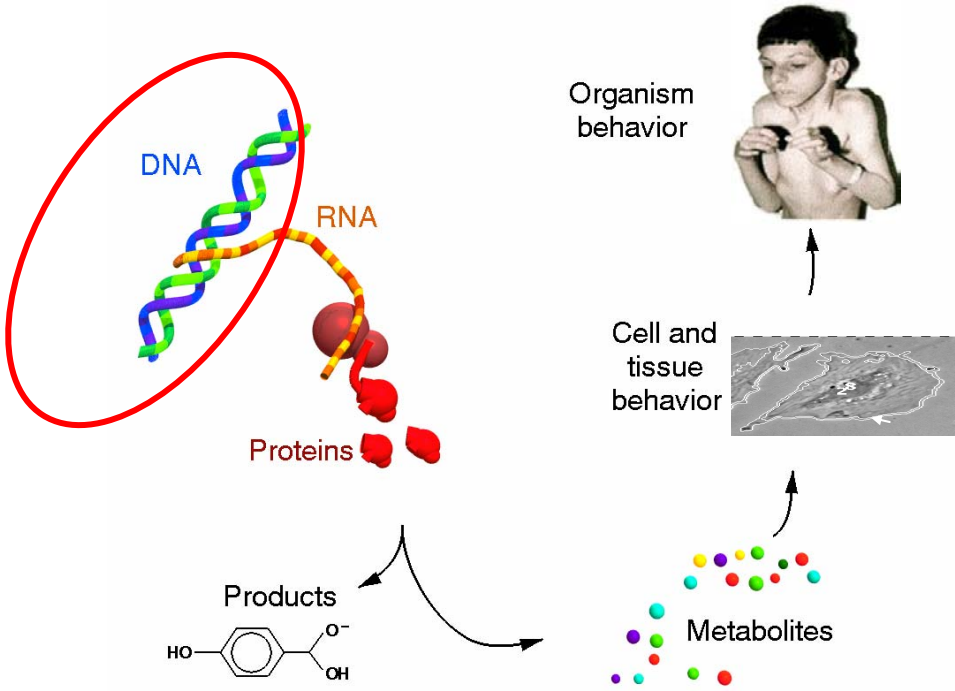

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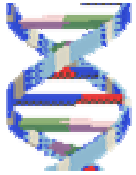
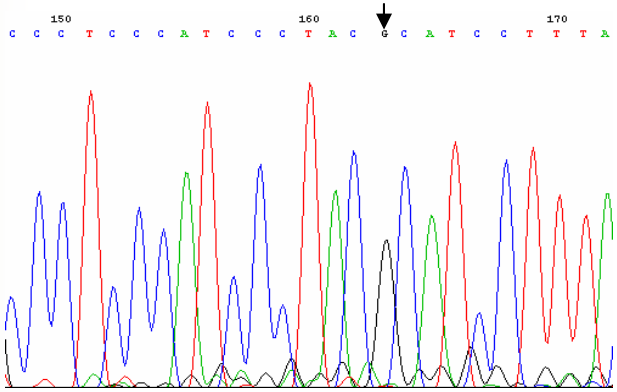
Metrology Needs in Bioscience

Gene Typing (Genotyping)



In disease diagnosis

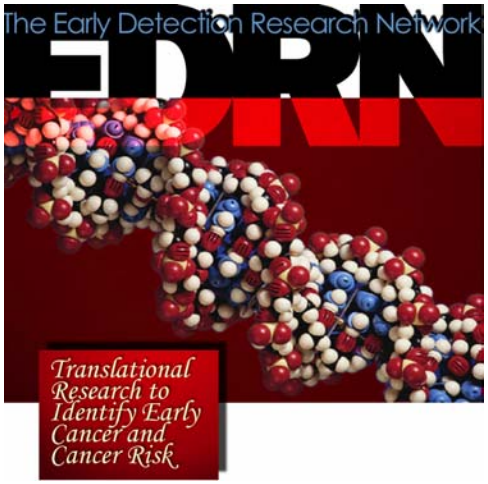
lung cancer disease marker



Oligonucleotide Sequence Determination

Red = T **Blue = C**

Green = A **Black = G**



NIST Biomarker Validation Lab
Early Detection Research Network
National Cancer Institute (NIH)

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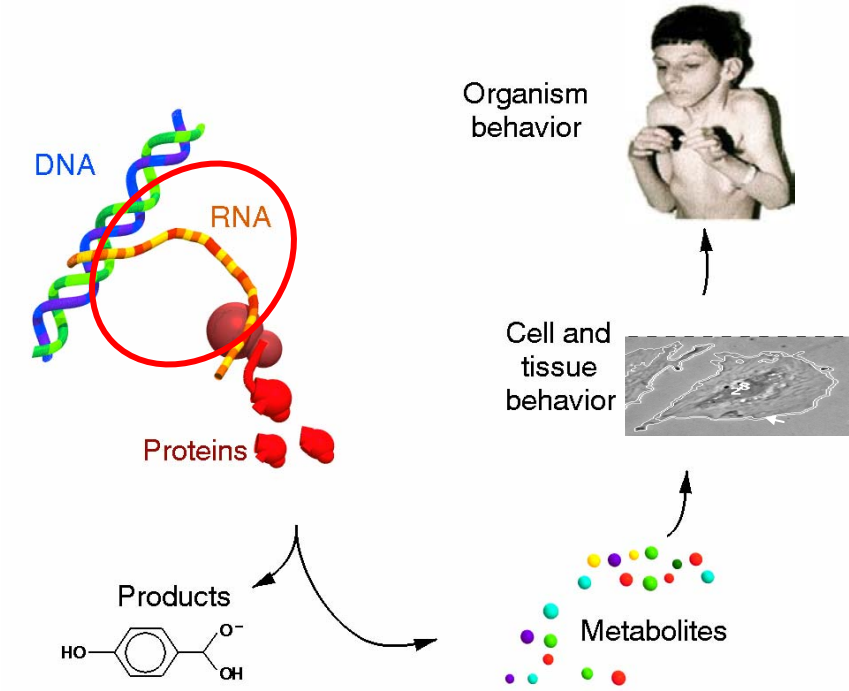

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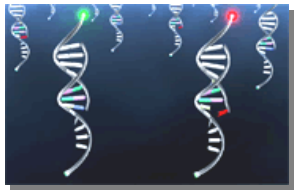
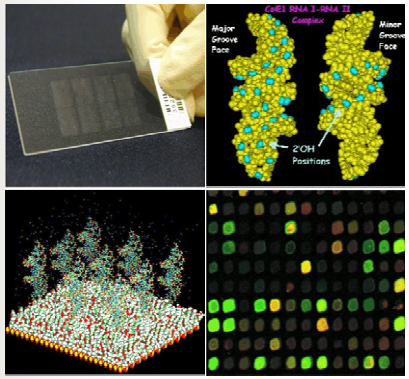
Metrology Needs in Bioscience

Gene Expression

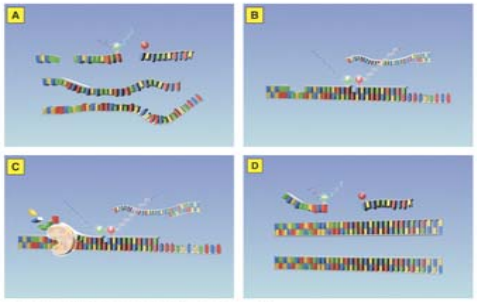


Hybridization of fluorescently labeled cDNA from RNA

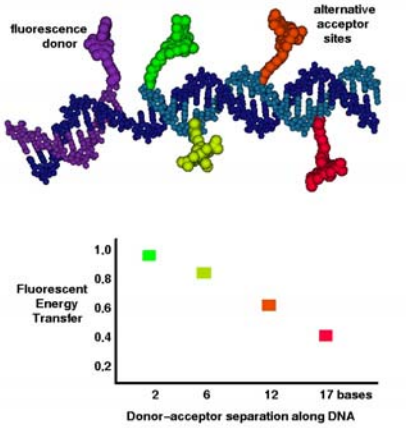
In disease diagnosis using gene arrays (e.g. microarrays, gene chips)



Labeled cDNA hybridizes to chip arrays – spots indicate presents of a gene, brightness indicates amount of gene product



Fluorescence Resonant Energy Transfer (FRET) indicates presence or absence of hybridized gene products



How DNA is labeled affects spot intensity!

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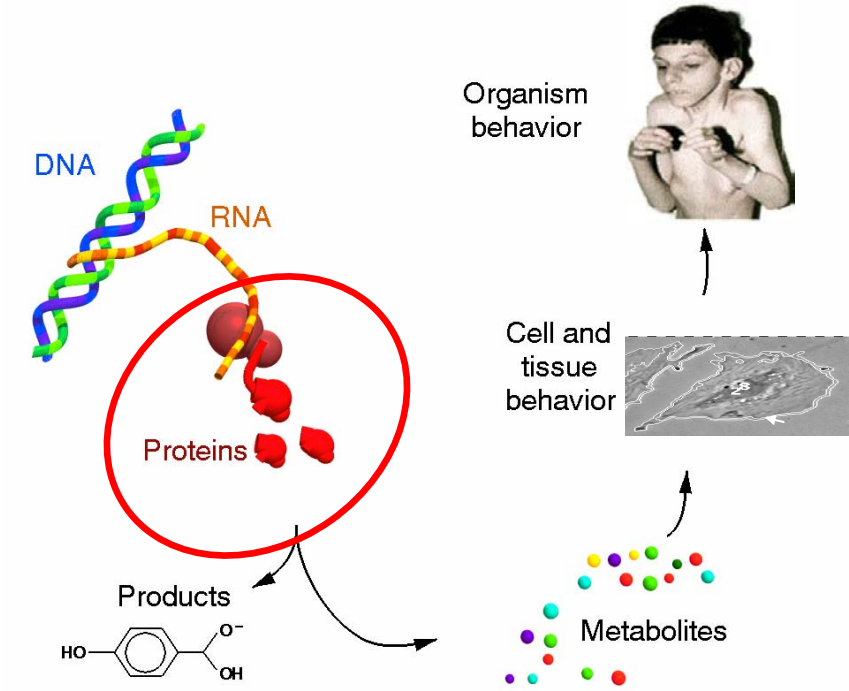

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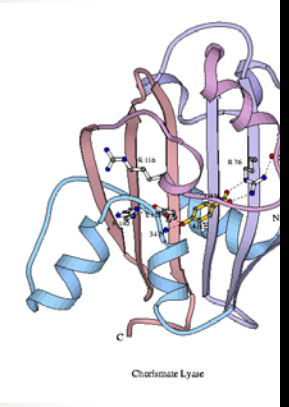
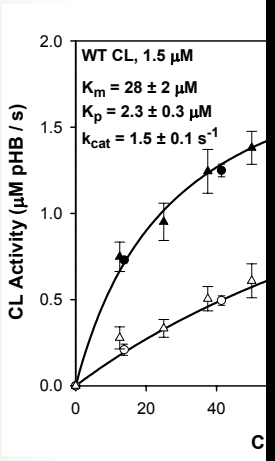
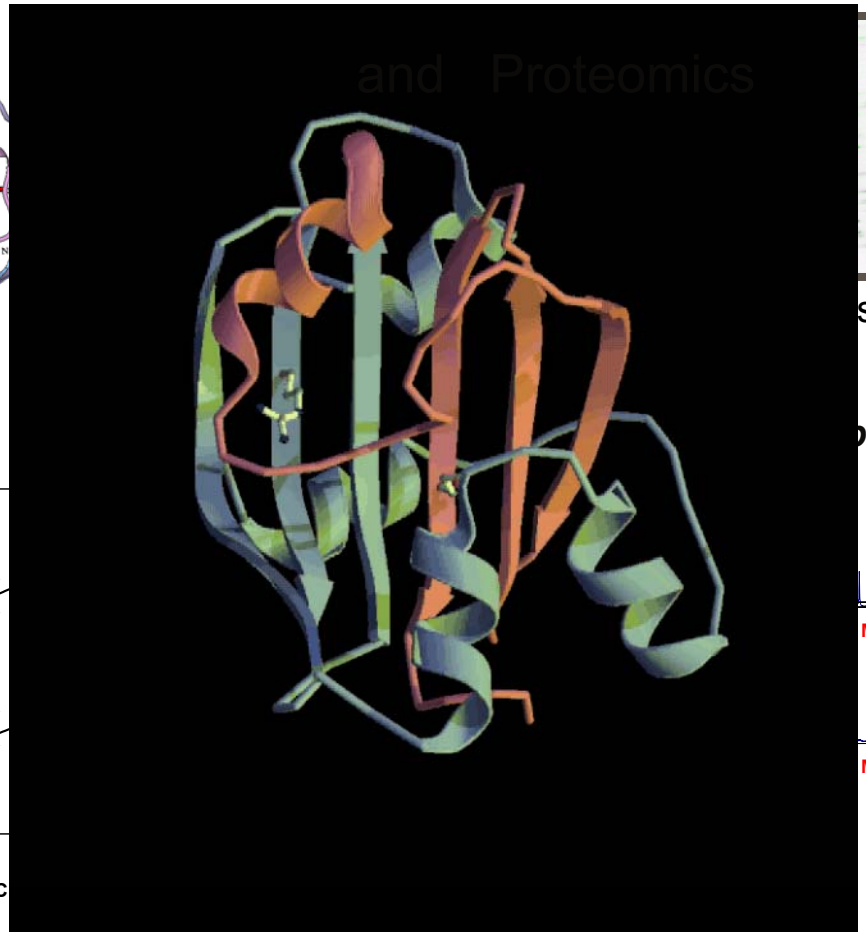
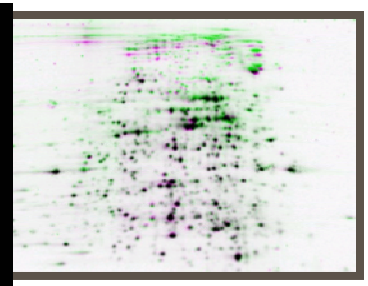


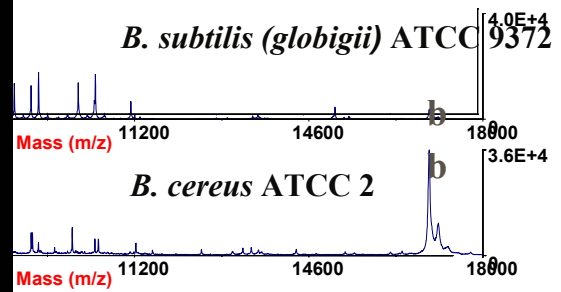






stem

ec of Cellular Digest



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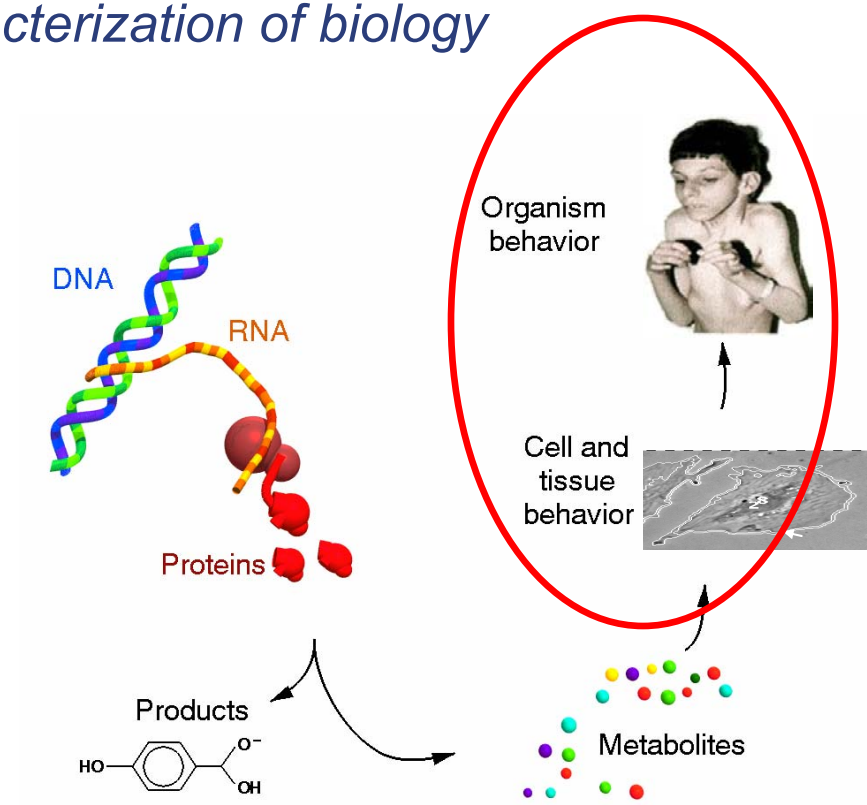

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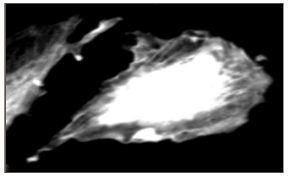
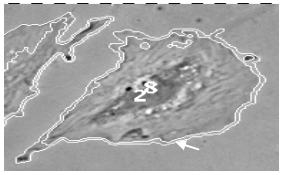
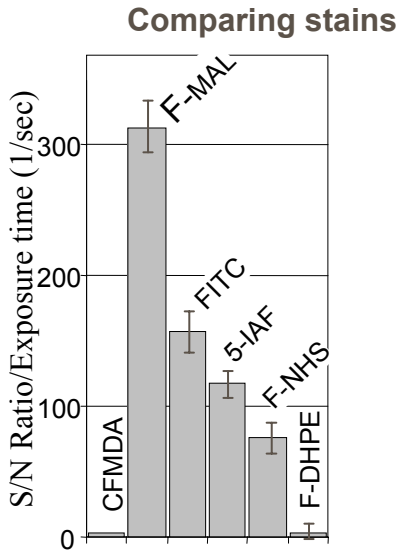
Metrology Needs in Bioscience

Cellular & Tissue Biology

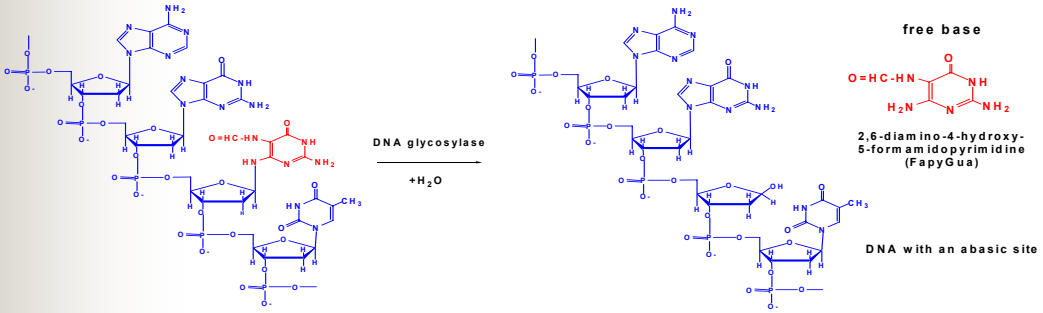
Imaging the morphology of smooth muscle tissue cells







DNA markers of oxidative damage



Cockayne syndrome sufferers have multisystemic disorders due to a defect in the ability of cells to repair DNA that is being transcribed.

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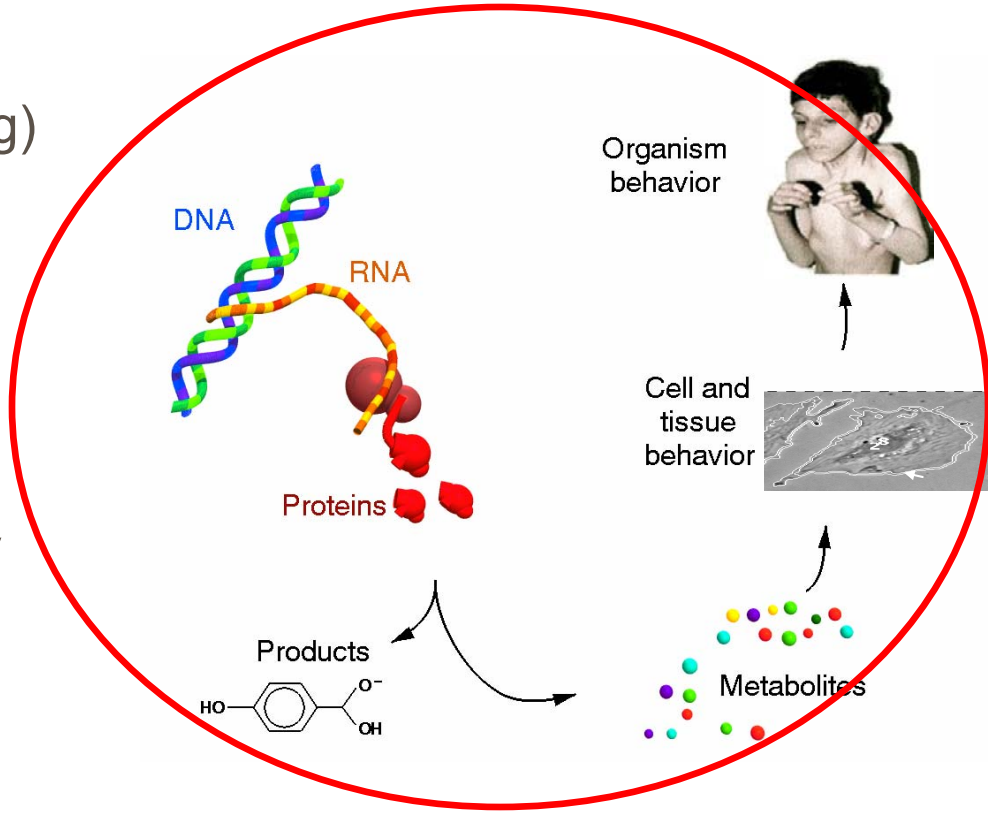

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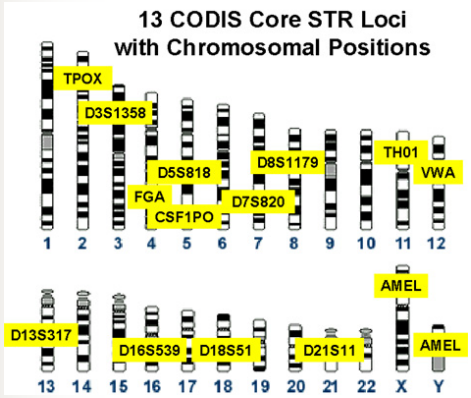
Metrology Needs in Bioscience

Bioinformatics

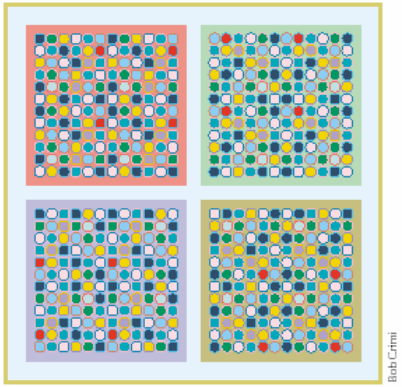
Genotyping

Short Tandem Repeat STRBase for Forensics



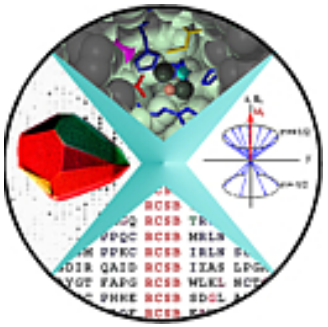
Gene Expression

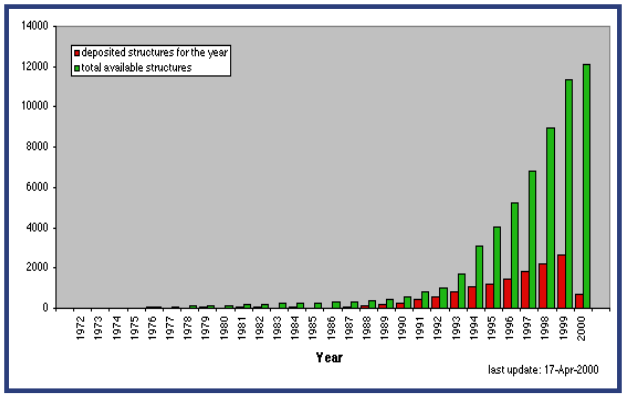
Microarrays and MIAME Convention for preserving pattern data



Proteins & Proteomics

Protein Data Bank





Cellular/Tissue Biology

Tissue specific biomarkers in the future.

Overview

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Current Activity: Health Care SRMs

Health Care Single Analyte SRMs

Marker	***Disease State***
Troponin-I	**Myocardial Infarction**
Glycated Hemoglobin	**Diabetes Status**
Homocysteine	**Risk of Heart Disease**
TSH	**Thyroid Function**
Speciated Iron	**Hemochromatosis**
PSA	**Prostate Cancer**
Folates	**Neural Tube Defects**
Under development	
Trinucleotide Repeat	**Fragile X Syndrome**
HER-2 neu	**Breast Cancer**
p53 DNA	**Breast Cancer**

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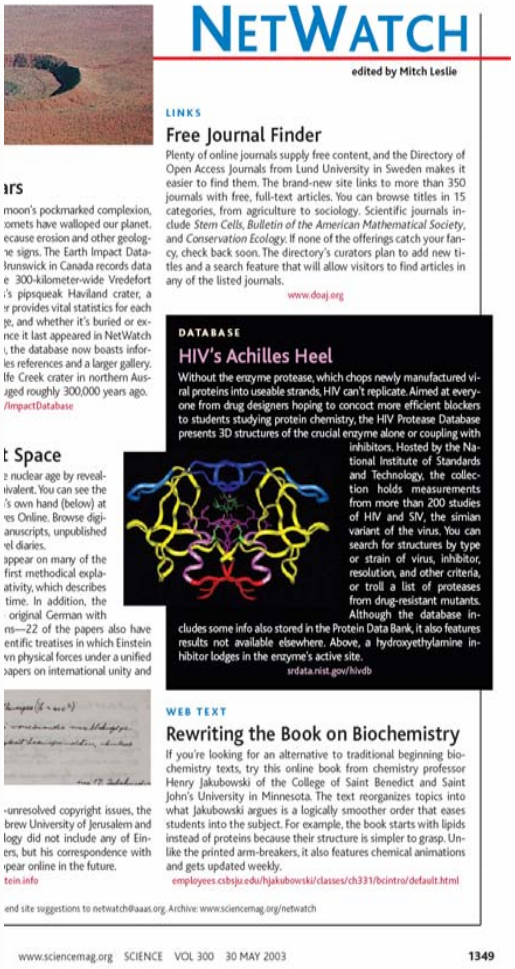
Current Activity: Fluorescence SRMs

- **SRM 1932 Fluorescein Solution**
  - **Certified for concentration and purity**
  - **Used for fluorometer calibration**
  - **Enables a NIST -traceable MESF fluorescence intensity scale to be established**

  (MESF = molecules of equivalent soluble fluorophore)

- **SRM 1933 Fluorescein Labeled Microbead Suspension**
  (*under development)*
  - **To be used for flow cytometer and suspension assay calibration (MESF intensity)**
  - **Will help meet the growing need for quantifying analyte per cell levels**

Current Activity: Databases



NETWATCH

edited by Mitch Leslie

LINKS

Free Journal Finder

Plenty of online journals supply free content, and the Directory of Open Access Journals from Lund University in Sweden makes it easier to find them. The brand-new site links to more than 350 journals with free, full-text articles. You can browse titles in 15 categories, from agriculture to sociology. Scientific journals include *Stem Cells*, *Bulletin of the American Mathematical Society*, and *Conservation Ecology*. If none of the offerings catch your fancy, check back soon. The directory's curators plan to add new titles and a search feature that will allow visitors to find articles in any of the listed journals.

www.doaj.org

DATABASE

HIV's Achilles Heel

Without the enzyme protease, which chops newly manufactured viral proteins into useable strands, HIV can't replicate. Aimed at everyone from drug designers hoping to concoct more efficient blockers to students studying protein chemistry, the HIV Protease Database presents 3D structures of the crucial enzyme alone or coupling with inhibitors. Hosted by the National Institute of Standards and Technology, the collection holds measurements from more than 200 studies of HIV and SIV, the simian variant of the virus. You can search for structures by type or strain of virus, inhibitor, resolution, and other criteria, or troll a list of proteases from drug-resistant mutants. Although the database includes some info also stored in the Protein Data Bank, it also features results not available elsewhere. Above, a hydroxyethylamine inhibitor lodges in the enzyme's active site.

srdata.nist.gov/hivdb

WEB TEXT

Rewriting the Book on Biochemistry

If you're looking for an alternative to traditional beginning biochemistry texts, try this online book from chemistry professor Henry Jakubowski of the College of Saint Benedict and Saint John's University in Minnesota. The text reorganizes topics into what Jakubowski argues is a logically smoother order that eases students into the subject. For example, the book starts with lipids instead of proteins because their structure is simpler to grasp. Unlike the printed arm-breakers, it also features chemical animations and gets updated weekly.

employees.csbsju.edu/hjakubowski/classes/ch331/bcintro/default.html

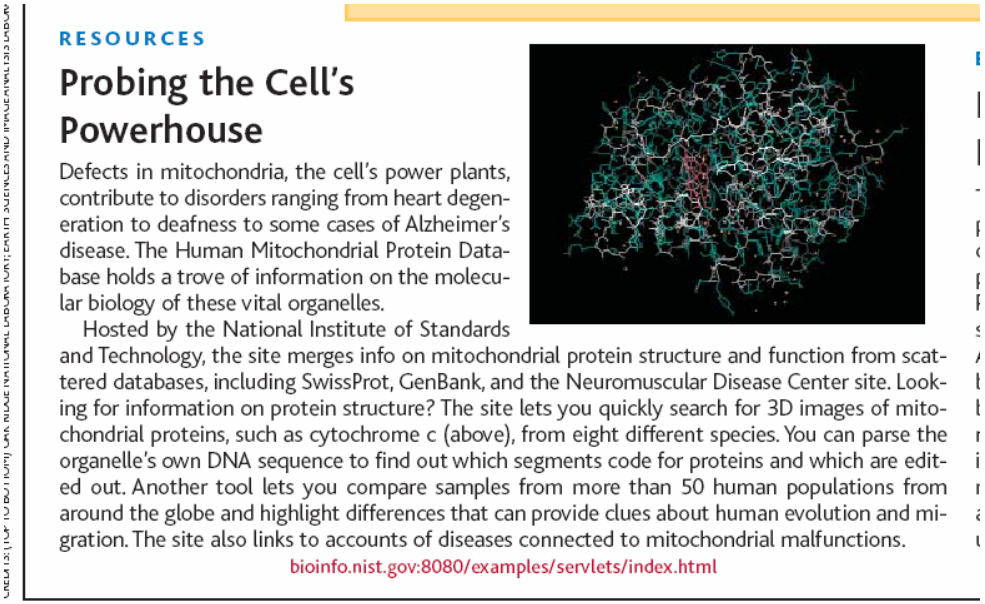
www.sciencemag.org SCIENCE VOL 300 30 MAY 2003 1349

← **Structural Database of HIV Protease**

Science, May 30, 2003

↓ **Human Mitochondrial Protein Database**

Science, May 2, 2003



RESOURCES

Probing the Cell's Powerhouse

Defects in mitochondria, the cell's power plants, contribute to disorders ranging from heart degeneration to deafness to some cases of Alzheimer's disease. The Human Mitochondrial Protein Database holds a trove of information on the molecular biology of these vital organelles.

Hosted by the National Institute of Standards and Technology, the site merges info on mitochondrial protein structure and function from scattered databases, including SwissProt, GenBank, and the Neuromuscular Disease Center site. Looking for information on protein structure? The site lets you quickly search for 3D images of mitochondrial proteins, such as cytochrome c (above), from eight different species. You can parse the organelle's own DNA sequence to find out which segments code for proteins and which are edited out. Another tool lets you compare samples from more than 50 human populations from around the globe and highlight differences that can provide clues about human evolution and migration. The site also links to accounts of diseases connected to mitochondrial malfunctions.

bioinfo.nist.gov:8080/examples/servlets/index.html

www.sciencemag.org SCIENCE VOL 300 2 MAY 2003

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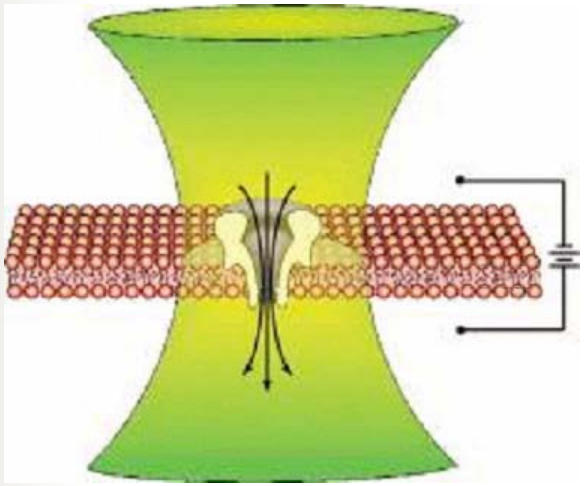
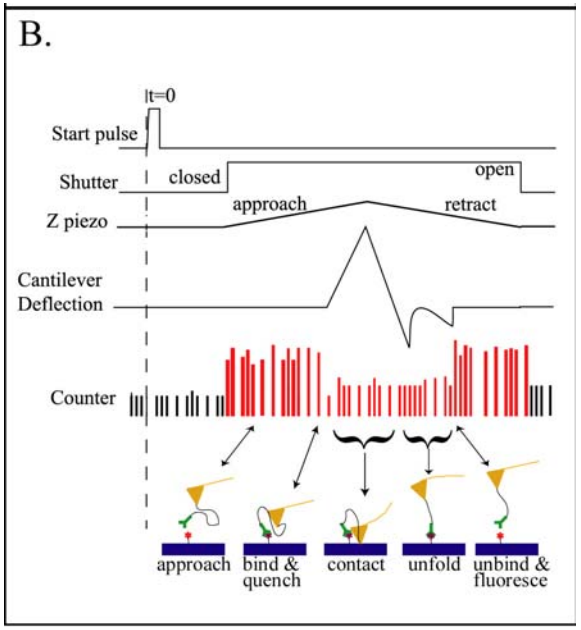
Current Activity: Advanced Measurements
Single Molecule Measurement & Manipulation (SM^3)

Single Molecule Detection Techniques:



Combined
Fluorescence Confocal Microscopy
& Ion Current Measurements

Simultaneous Force and Optical Measurements of Antibody/Antigen Interaction

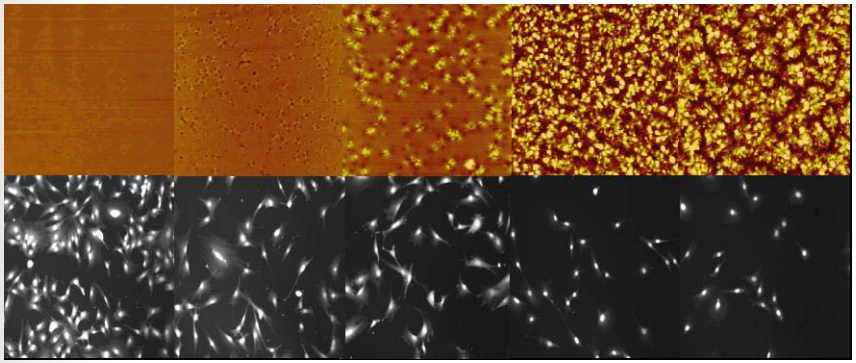


Fluor-labeled (Alexa 488) antibody attached to a glass slide is probed with an anti-Alexa antigen attached to AFM tip.

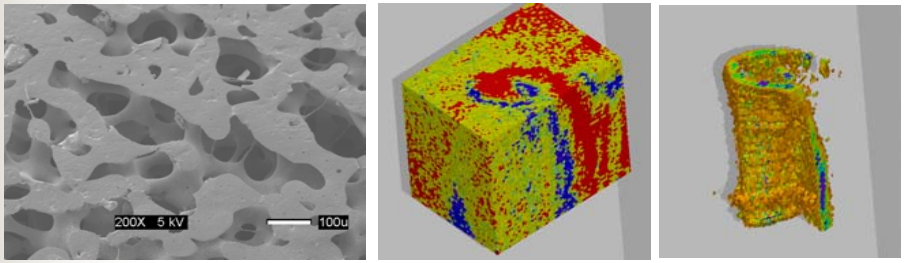
Current Activity: Advanced Measurements

Biomaterials for Tissue Engineering

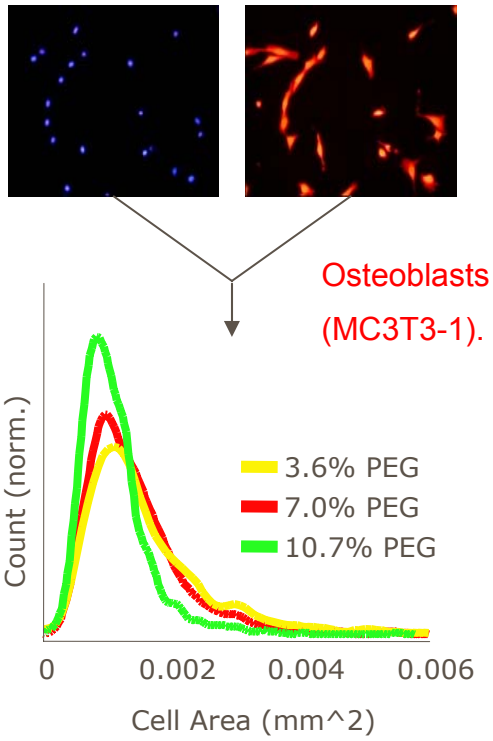
High-throughput Investigations of Cell-Material Interactions



Chemically Specific 3D Imaging of Tissue Development in Polymeric Scaffolds



Single-cell statistics



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Current Activity: Assessing Needs

- ***Industry*** – BIO, direct interactions, …
- ***Scientific Community*** – ACS, ASBMB, ABRF, IUPAC, FASEB, ACA, ASM, …
- ***Government Agencies*** –**NRC**, FDA, CDC, NIH, NIJ, DOD, USDA, OSTP/BRWG …
- ***Standards Activities*** – ASTM, NCCLS, CAP, **CCQM/BIPM** …
- ***Workshops*** – Fluorescence, Crystallography, CASP, AFM, Proteomics, Biotech Grains, Gene Expression & Microarrays…

Current Activity: DNA Standards for Forensics

1985 First human DNA profiling system established in U.K. (RFLP)

1987 DNA evidence first successfully used in the U.S.

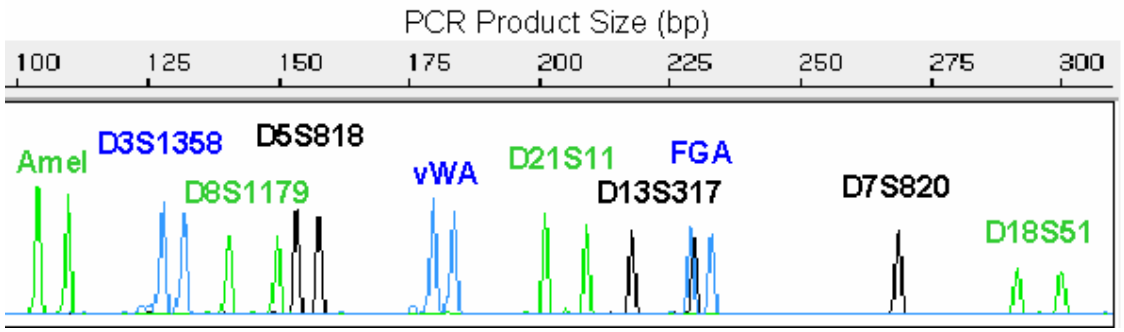
1991 Congress calls upon NIST to develop standards for DNA testing

Role for NIST: providing DNA standards for the forensic community – *standards will evolve* with advancements in *DNA typing technology*

1993 Commercial PCR based (STR) assays established

Current Activity: DNA Standards for Forensics

- SRM 2390 for RFLP-Based Profiling Standard (1992)
- SRM 2391 PCR-Based DNA Profiling Standard (1995)



- STRBase established (1997) – reference database with forensic STR info (http://www.cstl.nist.gov/biotech/strbase/)
- SRM 2392 Mitochondrial DNA Standard (1999)

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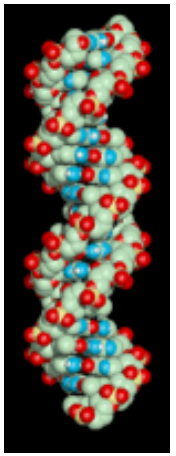
Current Activity: DNA Standards for Forensics

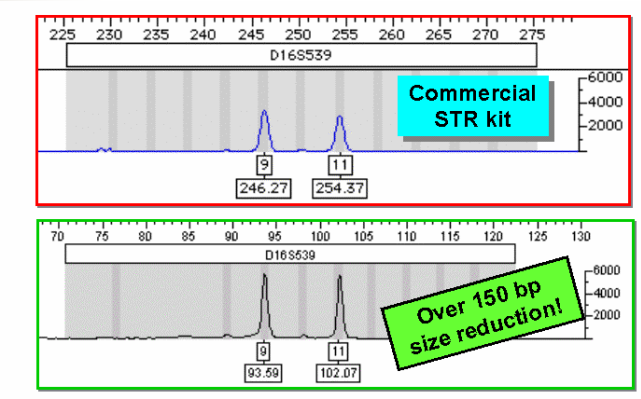
- Coordinate Interlaboratory Studies to independently evaluate laboratory performance
  - Latest study correlating DNA quantitation accuracy to STR multiplex intensity was published May 15, 2003 in *Analytical Chemistry*



- FBI DNA Advisory Board Standards issued in 1998
  - Standard 9.5: "The laboratory shall check its DNA procedures annually or whenever substantial changes are made to the protocol(s) against an appropriate and available NIST Standard Reference Material or standard traceable to a NIST standard."
- Member of FBI's Scientific Working Group on DNA Analysis Methods (SWGDAM) – help set validation guidelines and establish core loci for national forensic database

Current Activity: DNA Standards for Forensics

- Development of miniSTRs for more sensitive STR typing: used for typing highly degraded WTC samples



- Evaluation of Y chromosome SNPs and STRs to enable selection of optimal loci for human identification
- Y chromosome DNA profiling standard (SRM 2395) completed May 2003 to enable growing forensic, paternity, and genetic genealogy use of Y chromosome
- Human DNA quantification standard(SRM 2372) under development

Current Activity: Gene Expression

Oct 2002 – NIST Meeting on Metrology and Standards for Gene Expression Technologies (Device Makers, Technology Users, Regulators)

Dec 2002 – NIST/Industry Workshop on Standards Needs for Microarrays (Device Makers)

March 2003 – NIST/Industry Workshop on Universal RNA Standards held at Stanford University (Device Makers, Reagent Makers, Technology Users, Regulators)

100 folks attended, 33 companies, FDA,CDC,NIH/NCI, NIH/NIEHS, 4 Universities

Current Activity: Gene Expression

Workshop on Universal RNA Standards Stanford University: Goals

- **Identify key measurement & data issues facing technology**
  - *short- and long-term*
- **Will all platforms (microarray only? or bioarrays?) benefit from identified issues**
- **Identify issues that belong to NIST *(applied biology)* or NIH *(discovery biology)***
  - *differences*
- **Can issues be resolved without NIST**
  - *why & why not (pros & cons)*
- **Should NIST resolve these issues**
  - *define role NIST should take*
  - *identify partnerships with NIST to address issues*
- **Will microarrays require FDA approval for P.O.C. and diagnostic market**
  - *identify industry (research and diagnostic) consequences of not addressing issues of metrology*

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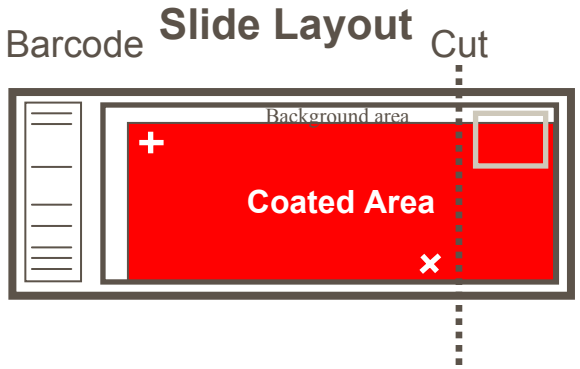
Current Activity: Gene Expression

Workshop Outcomes: Standards Activities
(what can we do, what should we not do)

- Disease Specific Devices/Reagents
  - Tied to IP, and therefore $$
  - Calibration should be part of the investment in technology development
  - No role for NIST
- Toxicogenomics
  - Role for NIST where platform and/or reagents will be used for a long time
  - Partner with FDA to define standards needs
- Data Transferability (over location, time)
  - One platform at a time, comment on life expectancy of platform
  - FDA/Industry consensus
  - NIST role in Phase 2
- Standard for Evaluating Lab Proficiency
  - Specific request directed to NIST by FDA

Current Activity: Gene Expression

- Microarray Slide SRM – 6 Device Makers
  - Size (active area, background area, placement)
  - Accommodation 1x3 and Affy format
  - Barcode
  - Thickness
  - Non-parallelism tolerance
  - Fiducial type and placement



- Standard for Quantitative PCR
  - Cross platform, unrelated to any known gene
  - First CCQM project, in collaboration with LGC, Ltd
- Particle Fluorescence SRM 1933
- Research on FRET
- Research on Non-Canonical RNA Structures

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Overview

- Economic/Societal Areas of Impact
- Metrology Needs in Bioscience
- Current NIST Biometrology Activity
- Looking to the Future

Biology at NIST: 20 years of Context

- NIST Base Programmatic Investment

  …….Competence in Biotechnology 1981, CARB MOU 1983, ……

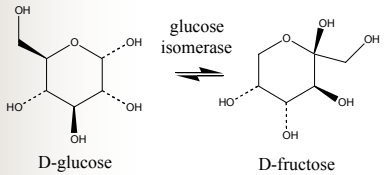
- External (Stakeholder) Investment

  …….NIH/RO1 Grant to CARB since 1987, NIJ 1993, NSF/DOE/NIH for PDB 1998,…….

- ATP Intramural Investment

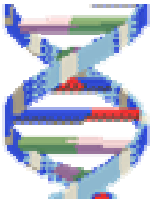
  ……..DNA Technologies 1993, Biocatalysis 1996, ……

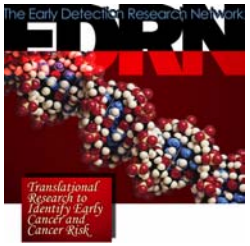
Biology at NIST: 20 years of Context

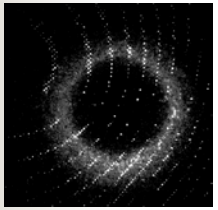


$\Delta G^{o} = f(\text{Temperature})$

↑ Yield = ↑ $

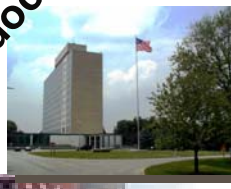






1980 1985 1990 1995 2000 2005 2010

1st Biotech Competence
CARB MOU
CARB Building
Biotech Division
NIJ/NIST DNA SRMs
PDB at NIST
NCI establish EDRN
SM³ & TE
NIST/NIH Directors Meet
NIST/NIH Postdoc Program



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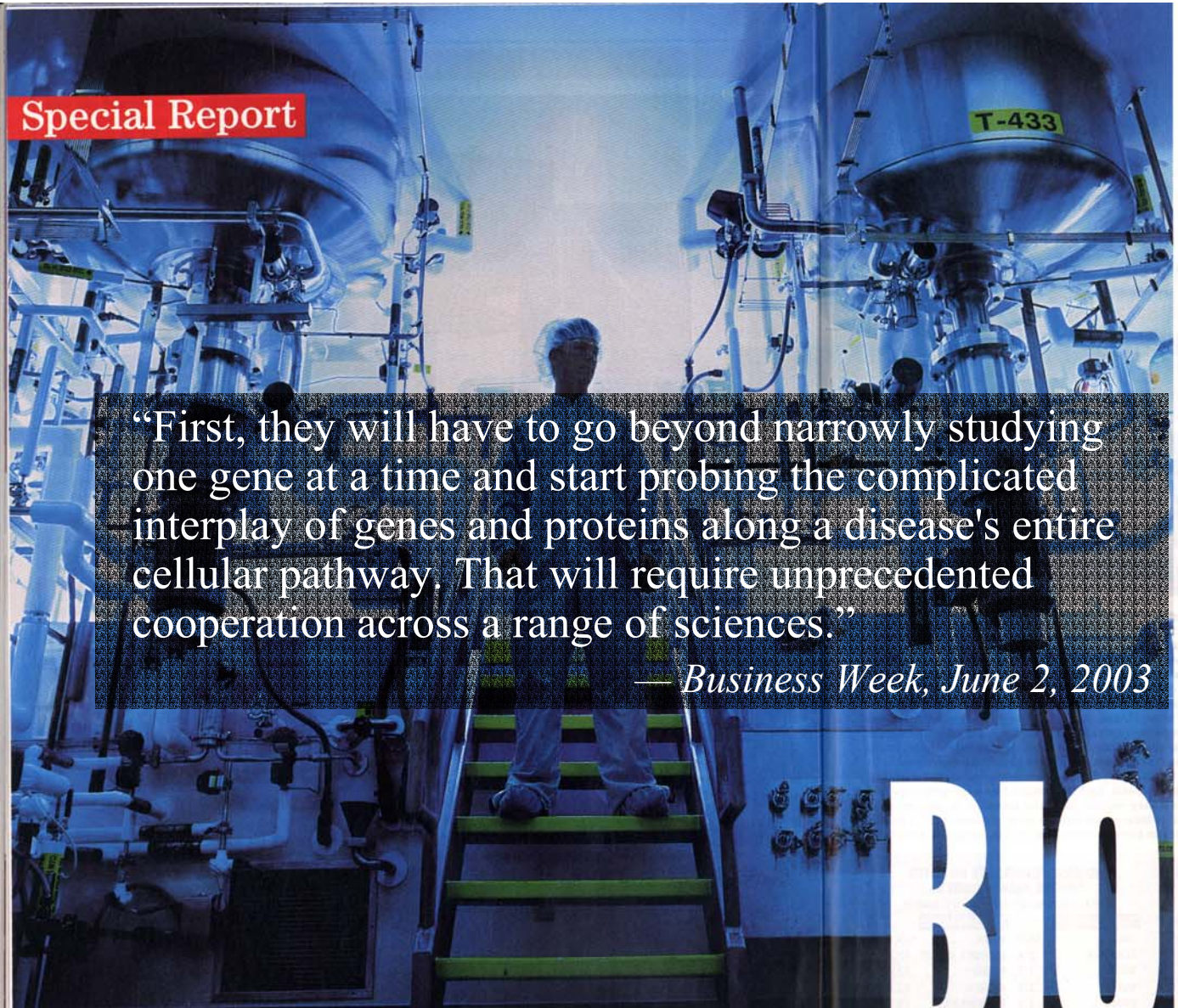
Looking to the future…



"First, they will have to go beyond narrowly studying one gene at a time and start probing the complicated interplay of genes and proteins along a disease's entire cellular pathway. That will require unprecedented cooperation across a range of sciences."

— Business Week, June 2, 2003

The science is hot and the stocks are up. Here's what's needed to deliver on the promise

BY ARLENE WEINTRAUB

Genentech's experimental colon cancer drug, Avastin, is anything but an overnight success. Thirteen years ago, one of its scientists found a gene that regulates blood flow to tumors, and started looking for a way to turn it off. It took the company five years to develop an antibody that could act as a switch in mice, and another three to fashion it into a drug. Then came animal tests, safety tests, and large-scale human trials to gauge the drug's effectiveness. And with every progress report, good and bad, Wall Street responded by pumping or pummeling the company's shares.

Right now, the pump is on. On May 19, Genentech Inc. announced that Avastin extends patients' lives when given with chemotherapy, raising hopes that it will be the first of a new class of drugs that can choke off the supply of blood to tumors. Investors pushed Genentech's stock up 45%, to $55, on the news. The trials aren't complete, and government approval isn't guaranteed, but Genentech is sanguine. "We're finding fundamentally different ways of treating people who are suffering and desperate," says Genentech Chief Medical Officer Dr. Susan Desmond-Hellmann.

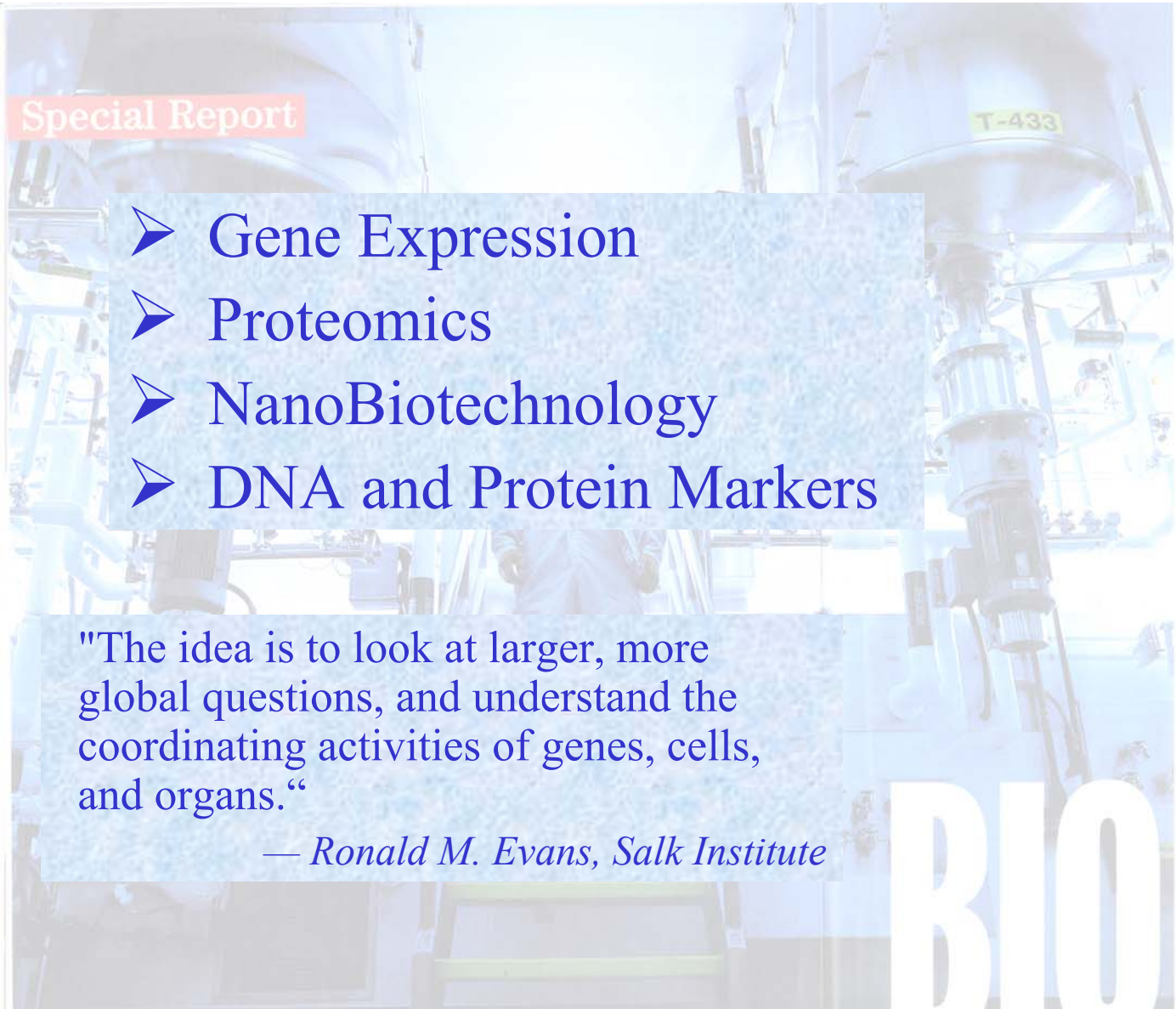
Desmond-Hellmann's optimism and her company's struggles with Avastin mirror the experiences of biotech execs everywhere. Scientifically, the industry has reached a watershed: The human genome has been sequenced, as have the genomes of some microbes and animals. Every day, scientists learn more about the intricate molecular dance of life and how the process runs wild in disease. The technology to transform these discoveries into life-saving therapies also is advancing quickly.

The medical and commercial rewards in biotech are now abundantly clear. While it will take months for Avastin to reach the market, analysts believe it could eventually pull in more than $1 billion a year. That would add nicely to the industry's revenue stream,

FIVE HURDLES FOR BIOTECH

RICHARD MORGENSTEIN

Looking to the future…



- Gene Expression
- Proteomics
- NanoBiotechnology
- DNA and Protein Markers

"The idea is to look at larger, more global questions, and understand the coordinating activities of genes, cells, and organs."

— Ronald M. Evans, Salk Institute